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**RECOLLECTIONS**

# Growing up in the golden age of protein chemistry

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## Prologue

More has been learned about biochemistry in the past half century than in all preceding time. Protein chemistry has been at the forefront of this revolution, but it did not emerge from the mystique of the era of colloid chemistry until physical methods for the characterization of proteins were developed in the 1930s, in particular, ultracentrifugation and electrophoresis. In 1938 a leading text of biochemistry (Gortner, 1938) stated "All of the reactions and interactions which we call life take place in a colloid system," and this credo was used to justify devoting a third of the text to colloids. Graduate students like myself studied up to 10 theories of protein structure, all of which proved to be wrong. For example, Svedberg, having developed the analytical ultracentrifuge, had dispelled the notion that proteins were heterodisperse colloids with no definite molecular weight or structure; yet, as late as 1940 he still proposed that molecules of most homogeneous proteins were simple multiples or submultiples of 34,500. Arne Tiselius's report of a method for protein electrophoresis had been rejected by the *Biochemical Journal*, but appeared in 1937 in *Transactions of the Faraday Society*, a journal no longer published (Tiselius, 1937).

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**Frank W. Putnam** is Distinguished Professor Emeritus of Molecular Biology and Biochemistry in the Department of Biology, Indiana University at Bloomington. His highly successful scholastic career started at Wesleyan University, where he received B.S. (Honors in Chemistry) and M.A. degrees, followed by a Ph.D. in Biochemistry from the University of Minnesota and an Honorary M.A. from Churchill College, Cambridge University. Major professional appointments include Instructor and Research Associate in Biochemistry at Duke University, Assistant and Associate Professor of Biochemistry at the University of Chicago, Professor and Head of Biochemistry at the University of Florida, and Professor of Molecular Biology and Biochemistry at Indiana University. Dr. Putnam is a member of the National Academy of Sciences and the American Academy of Arts and Sciences and is a recipient of many honors. He has served on many national and international committees, as well as on editorial boards of several prestigious journals. He was editor of a seven-volume treatise (two editions) of *The Plasma Proteins*, which has served for many years as a standard reference.

Professor Putnam is best known for his seminal contributions to the structure and function of immunoglobulins and other plasma proteins. He is continuing his research as an Emeritus Professor, and the present article describes part of his eminently successful career in protein science.

At this time I was an undergraduate chemistry major at Wesleyan in Connecticut and was enamored with the promise of colloid chemistry. My classmate Albert Lehninger and I were doing honors research on colloids under Professor Charles A. Hoover. Fresh from his Ph.D., Ross A. Gortner, Jr. had come to Wesleyan to initiate a course in biochemistry, then a novel area for a liberal arts college. After we graduated in 1939, Lehninger went to Wisconsin, which had the largest graduate enrollment in biochemistry, but I stayed on for a year to get an M.A. for the study of light scattering by colloids. One highlight of that period was a divisional meeting of the American Chemical Society at Harvard. I still remember the excitement of meeting John Edsall and observing Alex von Muralt's demonstration of double refraction of Edsall's muscle proteins. E.J. Cohn was a formidable figure whose brusque manner deterred me.

In 1940 I went to Minnesota to study proteins under Ross A. Gortner, Sr.; I was still excited by colloids and took courses under Herbert Freundlich, the great colloid chemist who had fled the Nazis. I remember Freundlich demonstrating "capillary chemistry" by showing the spreading ring of an extract of red cabbage; a similar experiment is now done in the fifth grade and is called paper chromatography. There it was right before our eyes! Not until A.J.P. Martin and Richard Synge recognized that chromatography could be done with colorless substances did it become a powerful tool in protein chemistry and biochemistry. As part of our doctoral research, George Schwert and I set up and used a Tiselius electrophoresis apparatus under the guidance of David Briggs and Gortner. At that time there were few such instruments in the world; there were no other satisfactory electrophoretic methods, and protein electrophoresis was an advanced art and a powerful new tool. Today, electrophoretic methods of varied types are found in most biochemical laboratories.

In the immediate prewar period Minnesota was a training ground for young protein chemists interested in learning physicochemical methods. One of the most brilliant was Lawrence Moyer. Tragically, he and my mentor Hoover were both killed in a blimp accident while doing secret wartime research off the New Jersey coast. Some

protein chemists who had earlier been at Minnesota returned in a lecture series, among them Hans Neurath, Henry Bull, and Max Lauffer. These lectures intensified my interest in proteins, but Pearl Harbor occurred and the nation was at war. Immediately after completing my Ph.D. thesis in June 1942, I left to do research on plasma proteins with Hans Neurath at Duke.

### Duke University, 1942–1946

Duke had a new medical school in the tradition of Johns Hopkins from whence many of the faculty had come. The Biochemistry Department headed by W.A. Perlzweig focused on vitamins at a time when nutritional deficiencies such as pellagra that had been rampant in the Southeast were just being wiped out by fortification of flour. Hans Neurath was an assistant professor, as was Phil Handler, who in later years became president of the National Academy of Sciences. At this time there was no commercial source of proteins or of apparatus suitable for determination of the molecular weight and shape of proteins. Biochemists had to prepare their own proteins and make their apparatus. With aid from the Rockefeller Foundation, Hans had set up one of the few laboratories capable of determining hydrodynamic properties of proteins, in this case by the methods of diffusion and viscosity. A Tiselius apparatus and an analytical ultracentrifuge were generously made available to me by the animal virologist, Joe Beard (known later as an early supplier of reverse transcriptase).

Hans and his group had made fundamental studies on the denaturation of proteins such as horse serum albumin and gamma globulin and had shown that regeneration (renaturation) led to a decrease in antigenicity. Our first wartime project was to study the denaturation and regeneration of bovine albumin, which because of its greater availability and naturally milder antigenicity might serve as a plasma substitute. We found that the antigenicity of regenerated bovine albumin was reduced by about 70%, but this was insufficient to produce a safe product. The need was soon eliminated because of development of the Cohn method for plasma fractionation and a national system of blood collection. However, this project aroused my interest in plasma proteins and immunochemistry, which became the lifelong focus of my research.

We next turned to study the interactions of synthetic detergents and proteins, such as serum albumin and sodium dodecyl sulfate, at a time when the acronym SDS was not yet a part of laboratory jargon. However, we soon were recruited into a national collaborative study of the antigenic basis of biologic false-positive reactions in serologic tests for syphilis. In the period prior to penicillin, this was a major problem for the wartime draft, and the project was sponsored by the Office of Scientific Research and Development. Hyperglobulinemic sera often gave false-positive tests, and one of my tasks was to perform electrophoretic analysis of such sera. A single analysis required a full day. I never forgot the variety of bizarre electrophoretic patterns given by sera from patients with multiple myeloma; this motivated my later research on Bence Jones proteins and myeloma globulins.

On the side we investigated the chemical and enzymatic properties of crystalline carboxypeptidase. Recently, as I discarded some 32,000 records of automated amino acid analysis of proteins and peptides, I recalled how tedious it was in 1945 to determine the release of a single amino acid from a peptide substrate using the gasometric ninhydrin method for free amino acids in the Van Slyke manometric apparatus. Mercury was needed to operate the Van Slyke but was unavailable in wartime. However, I gleaned enough from the gutters and drawers of a lab vacated by a clinical chemist, who was later diagnosed as having mercury poisoning. I believe our paper on carboxypeptidase was the first of the long historic series on proteolytic enzymes that issued from the laboratory of Hans Neurath.

In 1946 I left Neurath's laboratory and was succeeded by George Schwert, who carried on the program on proteolytic enzymes. However, for a short time I stayed on at Duke to investigate the molecular weight and homogeneity of crystalline botulinum A toxin. I was a civilian scientist attached to Camp Detrick at Frederick, Maryland, then the site of research on biological warfare for the Chemical Warfare Service of the Army, and now the site of the Frederick Center for Cancer Research. This crystalline toxin, the most potent known, contains about $220 \times 10^6$ mouse $LD_{50}$ per mg of nitrogen. Although the research was initially highly classified, a paper had appeared announcing that two groups working independently at Detrick (one for defense, the other for offense) had crystallized the toxin. I determined the electrophoretic and molecular kinetic properties of the toxin at Duke and Detrick and reported the results and also the amino acid composition at the Federation meeting in Chicago in 1947. Erwin Brand was in the audience and vigorously challenged the latter data; for at that time a separate microbial assay had to be done for each amino acid, chiefly by methods developed by Brand, and he thought no other laboratory had the capability. However, Camp Detrick had microbiological facilities and expertise unimaginable elsewhere at the time. Yet, I was put off by the military bureaucracy, and the objectives were obsolete in peacetime, so I left for Chicago. Currently, botulinum A toxin is used experimentally at high dilution in the treatment of some neurological disorders; in fact, my daughter was a recent patient.

### University of Chicago, 1947–1955

In 1947 Earl Evans, chairman of the Department of Biochemistry at the University of Chicago, gave me a post as a research assistant professor in a project on the bio-

chemistry of *Escherichia coli* bacteriophages. Isotopic study of intermediary metabolism, which had been initiated at Columbia by Schoenheimer, where Evans had trained, was opening up new pathways. Evans had recruited Schoenheimer's student, Konrad Bloch, and others from Columbia. Al Lehninger, Elwood Jensen, and Paul Talalay worked across the courtyard with Charlie Huggins. Many great physicists from the Manhattan Project including Leo Szilard, Enrico Fermi, and James Franck had stayed on across the street in the Biophysics Institute and were developing an interest in biology. Among the chemists were Frank Westheimer, who had Dan Koshland as a graduate student, and Harold Urey, whose student Stanley Miller published a landmark paper on prebiotic synthesis of amino acids. Larry Bogorad was in Botany studying chlorophyll biosynthesis. The Argonne Cancer Research Hospital under Leon Jacobson offered unexcelled facilities for human studies with radioisotopes. Isotopes not yet commercially available were provided by the cyclotron across the street. Best of all were the great graduate students, many of whom had come back from the war. Among them were Eugene Kennedy, Morris Friedkin, Charles Gilvarg, Irwin Rose, and Eric Conn. Students who worked with me included Lloyd Kozloff, Arthur Koch, and Eugene Goldwasser. It was a time of unique ferment and great opportunity in biochemistry.

I vividly recall a private conference on the minimum number of light quanta required for photosynthesis. The brilliant but arrogant Otto Warburg had come to the United States to work on this in the laboratory of Robert Emerson at Urbana in order to disprove Emerson's value of up to eight quanta per molecule of $O_2$ evolved. Then he came to Chicago for a conference with Franck and Hans Gaffron, who also worked on photosynthesis. Warburg postulated that the minimum number was four per $O_2$ molecule, but he had earlier reported a higher experimental value. The two Nobelists sparred for advantage. With feigned pain, Warburg chided Franck for having reneged on his earlier support of Warburg's theory. Franck replied that of course he believed it at first: "For Warburg is Warburg, and he is the son of Warburg." The point was that the results depended on the measurement of light, and Warburg's father (Emil Warburg) was an eminent physicist, a founder of quantitative photochemistry, and a teacher of Franck.

## The Phage Group

My initial assignment was to attend what was perhaps the first Phage Meeting at Vanderbilt where Max Delbrück was an associate professor. Only about 15 people attended, including Salvador Luria and his research associate Renato Dulbecco from Indiana; also present were Seymour Cohen, Al Hershey, Tom Anderson, Mark Adams, and Gus Doerrman—the very founders of the Phage Group. Harassed, yet stimulated by the tart criticism of Delbrück, Luria and Dulbecco worked out the theory of multiplicity reactivation of UV-treated phage on the blackboard. I was a novice and understood little, but in typical fashion Delbrück unexpectedly asked me to summarize the proceedings at the end of the meeting. One had to grow up fast in the Phage Group!

Delbrück and Luria believed that the riddle of the gene would be solved by combining genetics and radiation biophysics in the study of phage reproduction, and they had little use for biochemistry. However, Seymour Cohen had begun to use $^{32}P$ in the study of phage replication, as we also did at Chicago. Dominated by Delbrück, the midwestern phage meetings were often held in Chicago at Site B (B for brewery) left over from the Manhattan Project. Among the luminaries attending were Szilard and Aaron Novick, who had just invented the chemostat, and Joshua Lederberg from Wisconsin. From Indiana came Luria and his student Jim Watson, and occasionally H.J. Muller.

It wasn't until the phage meeting at Cold Spring Harbor in 1950 that biochemical studies of phage reproduction got respect. In 1947 Seymour Cohen had initiated biochemical studies of $^{32}P$ uptake during viral synthesis and had shown that most of the phage phosphorus was derived from the medium after infection. Shortly afterward, we also began study of the origin of viral phosphorus. In a 1950 paper with the subtitle "The fate of the infecting virus particle," Kozloff and I, using $^{32}P$-labeled T6 phage, showed that the original infecting phage particle does not survive infection but does contribute about one-third of its phosphorus (i.e., DNA) to its progeny (Putnam & Kozloff, 1950). This was the predecessor of the famous Hershey–Chase experiment that showed that injection of viral DNA into the host bacterium is the critical act of phage infection. Watson has remarked that he was "... most affected by the talk of Kozloff and Putnam on their failure to observe 100% transfer of parental phage $^{32}P$ to the progeny particles" (Cairns et al., 1966). Later in 1950, Delbrück held a conference at Caltech that was continued around the camp fires during a 3-day trip through Death Valley. I recall Delbrück and Herman Kalckar climbing up the unstable rocks until Delbrück urged Kalckar to cease, for the Dane had sustained a fracture in an earlier climb. The 1950 conferences changed Delbrück's attitude on biochemical studies and also Luria's. They sent Watson to Denmark to study nucleic acid chemistry with Kalckar (Cairns et al., 1966; Olby, 1974). Watson later worked with Ole Maaløe on a project that had originated in our group at Chicago but became bored with it and moved to Cambridge (Olby, 1974).

By 1950 my interests began to return to plasma proteins. The bizarre electrophoretic patterns of myeloma sera remained vivid in my mind and became more intriguing as I began to study sera from new patients. Charlie

Huggins gave me some Bence Jones proteins; I was struck by the variety in molecular and electrophoretic properties of these proteins (now known to be light chains of immunoglobulins). A structural approach to this puzzle occurred to me when I read a paper by Rodney Porter, who had worked for his Ph.D. with Fred Sanger on the free amino groups of hemoglobins (Porter, 1950). (At that time one could publish a paper just on such data! Compare that to today when complete sequences of proteins or DNA are rejected as not being novel.) Using Sanger's dinitrophenyl (DNP) method, Porter had found that the amino-terminal groups of some animal gamma globulins gave multiple nonstoichiometric values. However, the amino-terminal pentapeptide sequence of nonimmune rabbit gamma globulin and that of rabbit anti-ovalbumin were identical, suggesting possible identity in primary structure of normal gamma globulin and antibodies. I resolved to apply this approach to Bence Jones proteins and myeloma globulins. As a Markle Scholar I had travel funds for a sabbatical, so I took my proteins and also T6 phage to Sanger's laboratory in Cambridge. There I showed that individual Bence Jones proteins from different patients differed in amino end groups, as did myeloma globulins (Putnam, 1953). I believe this was the first evidence for variability in sequence of what were later called light chains and immunoglobulins. I discarded the T6 phage and never worked on phage again.

**Cambridge, 1952–1953**

Three times I have been fortunate to participate in highly interactive, intellectually stimulating groups that made a difference in science: the Phage Group, the Cambridge group at the time of the Double Helix, and the Chain Gang of antibody fame. Absent these experiences, my research might have been mundane. Cambridge in 1952 was in ferment. Sanger, having completed the sequence of insulin in the confines of the Protein Hut with one associate for the B chain (Hans Tuppy) and one for the A chain (E.O.P. Thompson), had just moved with one assistant to the main building of the Sir William Dunn Institute of Biochemistry. There I learned why glacial acetic acid (freezing point 16.7 °C) is called glacial, for it froze when the heat was turned off during holidays. In 1952 meat and coal were tightly rationed in Britain. We had a hard time heating our flat in Bottisham Hall, where we had followed our Chicago neighbors, Al and Jan Lehninger, and were succeeded by Bill and Ingeborg Harrington.

Sanger's determination of the amino acid sequence of insulin was an historic achievement because it provided the first proof that proteins had a unique primary structure; it also set the strategy for protein sequence analysis. This period is best described by Sanger in an autobiographical chapter (Sanger, 1988). In those days before automated amino acid analyzers and protein sequenators, methods for protein sequence analysis were primitive, and none existed for nucleic acid sequencing. In fact, Sanger told me that he had determined the insulin sequence by a strategy that involved overlapping alignment of a series of DNP-peptides without ever doing any quantitative amino acid analysis. For that he had depended on published composition data and the fact that insulin contained only one lysine residue. Indeed, not long before, Stanford Moore had been working in the Protein Hut trying to refine the colorimetric ninhydrin analysis for amino acids that were separated by chromatography on starch columns and collected in test tubes with a fraction collector. The complete analysis of a protein hydrolysate required a week and more than 300 manual ninhydrin analyses. A.C. Chibnall's group was still in the Protein Hut and was active in protein analysis; Kenneth Bailey was working on myosin. In chemistry in the laboratory of Alex Todd (now Lord Todd of Trumpington), great strides were being made by Gobind Khorana and others in the synthesis of nucleotides. However, there was little interest then in DNA among the Cambridge biochemists; other than proteins, their primary concern under Frank Young was with hormones and intermediary metabolism. I believe I gave the first seminar on phage in the Sir William Dunn. Afterward Fred Sanger came up to me and said, "What are you working on that stuff for?" Of course, he later developed the method now most used for DNA sequence analysis and won his second Nobel Prize.

Paper chromatography had been developed by Martin and Synge, who won the Nobel Prize in 1952. On his return from Stockholm, Dick Synge stopped by Cambridge to see Sanger, and Rodney Porter came up from Mill Hill. There was a great reception for Synge at Trinity, his old college, to which Sam Perry took me. I remember remarking to the historian and former Master, Sir George Trevelyan, that he must be proud of Synge for having won the Nobel Prize. I was abashed when Trevelyan replied he didn't know whether Synge was the 12th or 13th Trinity man to receive the Nobel Prize because he didn't know how to count Bertrand Russell, who had received the Prize in literature. (Trevelyan did not mention that the famed mathematician Bertrand Russell, who was a leading pacifist during World War I, had been dismissed from his lectureship at Trinity in 1916 and imprisoned for sedition, although he was restored to his lectureship in 1944.)

Across the way in the Cavendish there was also great ferment, but it was focused on both proteins and nucleic acids. Max Perutz was trying to determine the structure of hemoglobin, and John Kendrew that of myoglobin. Vernon Ingram was there, yet it would be another 5 years before he showed that sickle-cell anemia was a molecular disease by determining the single amino acid difference of normal hemoglobin and sickle cell hemoglobins by fingerprinting. As is described in their classic books (Watson, 1980; Crick, 1988), in the fall of 1952 Francis Crick and Jim Watson were still frantically trying to solve the

structure of DNA before Linus Pauling did. In this they had much help from Jerry Donohue. On occasion, Jim came to see me in Sanger's laboratory; once after his visit Fred asked, "Who is that Irishman who comes to see you?"

This was the era of the Double Helix, in which I had only a small part. It was a time of intense work and intellectual stimulation, relieved by partying with Francis Crick and the visiting postdoctorals from America and the "colonies" (i.e., Canada and Australia). There were also practical jokes and episodes not described in *The Double Helix* (Watson, 1980) and better left untold; not the least was the jockeying that prompted the simultaneous appearance in the April 25, 1953, issue of *Nature* of papers by the Wilkens group and by Franklin and Gosling immediately following the paper of Watson and Crick. The importance of their discovery was made clear by the famous paper of Watson and Crick (1953) that followed shortly, but few then could discern the far-reaching implications for genetics and molecular biology. At first, like many others, I was a bit skeptical, for I was aware that neither Crick nor Watson knew or cared much about biochemistry. Yet, I was soon convinced and knew it was for the gold. Indeed, Crick recalls that I was the first to suggest to him that it would bring the Nobel (Crick, 1988).

## Return to Chicago, 1953–1955

On my return to Chicago I gave the graduate course in biochemistry. On describing the Shemin and Rittenberg classic study of the life span of the red cell, I recognized that there had been a small incorporation of $^{15}N$ into the plasma proteins (Shemin & Rittenberg, 1946). At that time, when isotopes were novel and obtaining approval for study of human subjects was less stringent, one could discover a metabolic phenomenon by a single experiment with one volunteer. (In this case the subject was David Shemin.)

On the basis of their paper I conceived of an experiment to study the biosynthesis of Bence Jones proteins, then thought to be a degradative product of nitrogen metabolism and possibly related to gamma globulin. With all the funds I could muster ($600) I commissioned Charles Gilvarg to synthesize glycine with 60 atom percent excess $^{13}C$. With the help of Steven Schwartz at Cook County Hospital, this was given orally to a patient with multiple myeloma. The Bence Jones protein, the albumin, and the myeloma globulin were purified from urine and blood samples. The proteins were hydrolyzed and the glycine separated chromatographically. The $^{13}C$ was measured in a mass spectrometer built by Herbert Anker, for none were then available commercially. We had hoped to get an incorporation of at least 0.2 atom percent excess, because that was the lower sensitivity level of the instrument. In fact, we did get this value for the albumin and globulin, but we got an incorporation almost 10-fold higher in the Bence Jones protein excreted the first day (Putnam & Hardy, 1955).

This was the most exciting experiment of my life, for it showed that Bence Jones protein was not a degradative product of tissue or plasma proteins, as had been thought, but rather it was rapidly synthesized and excreted. Some were at first skeptical of this result, but we repeated it with $^{15}N$-glycine and $^{14}C$-glutamic acid in other patients and showed that metabolically labeled Bence Jones protein was excreted within 15 min after intravenous injection of labeled amino acid. We concluded that Bence Jones protein was an abortive product of gamma globulin synthesis. The great puzzle was how or why patients with multiple myeloma could synthesize individually specific proteins not identifiable in healthy individuals. I spent the next decade trying to solve this puzzle. The answer came only when the structural variability of light chains and immunoglobulins was elucidated by protein sequence analysis, in part by my laboratory.

## The 1955 Gordon Conference

As Chairman, I organized the 1955 Gordon Conference on Proteins and Nucleic Acids (for at that time both subjects were covered in a single meeting). This was the beginning of the era of protein sequence analysis and of the search for the Holy Grail of the genetic code. I almost canceled the meeting because the conference director tried to withhold travel funds I had raised for invited speakers. Although amino acid analyzers were not yet commercially available and protein sequencers not even dreamed of, sequence analysis was being done by paper chromatography and electrophoresis. Also, Stein and Moore were developing sequence analysis by stepwise Edman degradation and automated amino acid analysis by column chromatography. Among the protein chemists participating were Fred Sanger, Rodney Porter, Chris Anfinsen, C.H. Li, and Klaus Hofman. Those gathering in an attempt to break the genetic code included Francis Crick, Jim Watson, Gobind Khorana, and George Gamov. The first night was dramatic. A report had just appeared in *Time* (1955) that C.H. Li had sequenced ACTH (corticotropin, a polypeptide of only 39 amino acid residues, but then a tremendous feat), however, there was no mention of the similar achievement by Paul Bell and associates at Lederle and by Will White at Armour. Klaus Hofman, chairing the session, held up the copy of *Time* while introducing Li, the first of the three speakers on ACTH. Everyone waited breathlessly for the still unpublished sequence. However, it turned out well, because the discrepancies were minor and attributable to species differences. One of the highlights of the conference was George Gamov doing card tricks to show that all 20 amino acids could be encoded by only four nucleotide bases. There were a few practical jokes, including cutting

of the rope for the school bell that wakened the conferees who had spent the nights drinking and debating. Mr. Mahan, the Headmaster, never forgave us. The Conference ended with many participants trekking to Woods Hole and converging uninvited on the home of Albert Szent-Gyorgy because some joker had issued a series of false invitations for a party there.

## Epilogue

In September 1955 I went to Gainesville, Florida, to found the Department of Biochemistry as the second member of the medical school faculty. Just as the origin of phage phosphorus had lured me earlier, so did the origin and role of Bence Jones proteins continue to tantalize me after the isotopic studies of their biosynthesis. I decided to undertake the formidable task of amino acid sequence analysis of Bence Jones proteins at a time when the complete sequences had been reported for only a few proteins, such as insulin, hemoglobin, myoglobin, and some pancreatic enzymes. To make matters more difficult, there were two kinds of Bence Jones proteins (now known as kappa and lambda light chains), and I had earlier shown that each type exhibited variability in sequence—at least at the amino-terminal end. Fortunately, Koiti Titani, an experienced protein chemist, came from Japan to work with me on this project. In the 1960s, protein sequence analysis could only be done by subtractive Edman degradation, that is, by stepwise removal of the amino-terminal amino acid of a peptide, followed by amino acid analysis of the residual peptide after each step. This required hundreds, and even thousands, of amino acid analyses. At one time we had three amino acid analyzers operating nearly full time (Fig. 1).

Interest in the structure of Bence Jones proteins, myeloma globulins, and normal gamma globulin was rapidly growing. The Chain Gang (also known as the Piece Corps) was forming to solve the Antibody Problem. It was a competitive yet congenial group that held informal meetings culminating in the famed Antibody Workshop in January 1965 at Warner Springs, California. The grunts in the laboratory such as myself had been invited to present

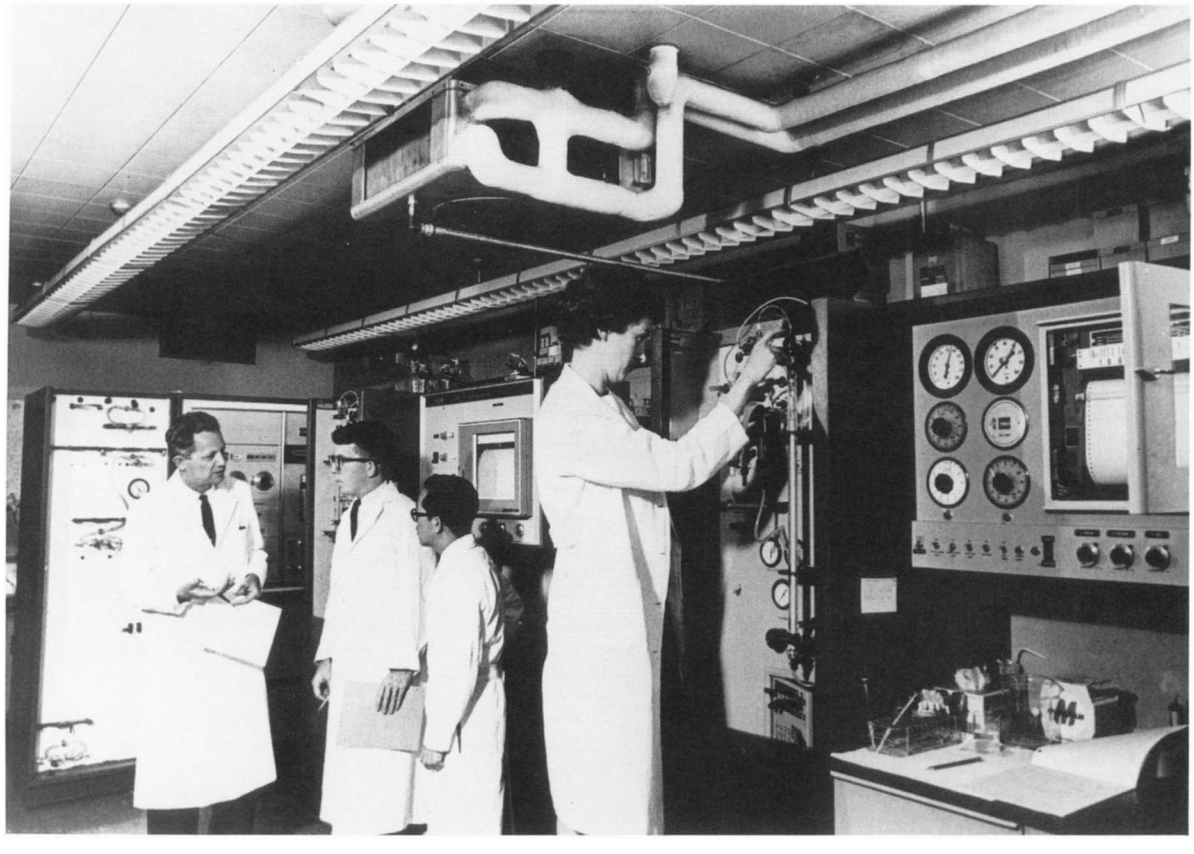

**Fig. 1.** Protein sequence analysis in the mid-sixties. Photograph taken in the laboratory of the author (far left) about the time the amino acid sequences of human kappa and lambda Bence Jones proteins (light chains) were completed using the method of subtractive Edman degradation. At that time, the amino acid analyzer was the workhorse of protein sequence analysis. Also shown are two laboratory assistants and Dr. Koiti Titani (second from the right). Dr. Titani was later a research professor in the laboratory of Dr. Hans Neurath and is now a professor at Fujita Health University School of Medicine, Aichi, Japan. He is on the Editorial Advisory Board of *Protein Science*.

their data, and the great brains to interpret it. There I presented evidence on the variability in sequence of Bence Jones proteins and was followed by Norbert Hilschmann. The results produced a sensation, the more so because Hilschmann flashed his slides and refused to reveal his data for examination prior to publication. His paper came out in the July *PNAS* (Hilschmann & Craig, 1965) and ours in an August issue of *Science* (Titani et al., 1965), and the great sequence race to determine immunoglobulin structure and the origin of antibody variability and specificity was on. Although much more work remained to be done, the chief clues to the paradox were revealed in the 1967 Cold Spring Harbor Symposium on antibodies and in the Nobel Symposium on gamma globulins that followed almost immediately at Södergarn near Stockholm, which was sponsored by Arne Tiselius. I had grown up in the Golden Age of Protein Chemistry and participated in the Flowering of Immunology. Now it was time for the *enfants terribles* of molecular biology to cut their teeth.

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